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# Serum concentrations of lipids and lipoproteins and their correlations with thyroid hormones in clinically healthy German shepherd dogs: Effects of season, sex and age

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## Abstract

Although there are many evidences regard to the relationship between serum thyroid hormones, cholesterol, triglyceride and lipoproteins in different animals, but there are limited references in dogs. The aim of this study was to determine the correlation between thyroid hormones and concentrations of serum lipid and lipoprotein profiles, and also to detect relationship between the measured parameters in different season, sex and age groups of dogs in Ahvaz district, Southwest of Iran, on 2011. Blood samples were taken from the cephalic vein of 60 clinically healthy and non-pregnant native dogs in two seasons (summer and winter) and in two age groups (<3 years and >3 years). Sampling was done only once from each animal (30 dogs in every season). The serum concentrations of cholesterol, triglyceride (T.G), very low density lipoproteins (VLDL-cholesterol), low density lipoproteins (LDL-cholesterol), high density lipoproteins (HDL-cholesterol and their correlations with thyroxine ($T_4$) and triiodothyronine ($T_3$) hormones were measured in the studied dogs. The mean serum concentrations of cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol (mmol/L), $T_4$ and $T_3$ (μmol/L) were measured in the present survey. The above parameters were also obtained in two summer and winter seasons. Season had a significant effect on T.G, HDL, LDL and VLDL levels in the studied dogs ($p<0.05$). The concentrations of serum $T_3$ and $T_4$ were lower in summer compared with winter season, without significant difference ($p>0.05$). No significant differences were detected for the measured cholesterol, $T_3$ and $T_4$ parameters in different age, sex and season groups ($p>0.05$). To the best of our knowledge, there was no previous research regarding the correlation of the thyroid hormones with the serum lipids and lipoproteins in dogs in Iran. Our results showed that thyroid hormones affect T.G, HDL, LDL and VLDL levels and observed seasonal change in serum may be partly due to changes in thyroid hormones.

**Keywords:** thyroxine ($T_4$), triiodothyronine ($T_3$), lipid, lipoprotein, dog, Ahvaz

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## Introduction

Although there are many reports on seasonal changes of thyroid hormones, lipid and lipoproteins in different animals, but there are limited references in pet animals including dogs. Studies have shown that species variations exist and that, even within species, significant differences may occur. The normal concentrations of serum lipids and lipoproteins have been reported in many animals including sheep, cow, horse, pony, reindeer calf, cheetah, camel and Iranian water buffalo (Nazifi *et al.*, 2007; Chatterjea and Shinde, 2005). Appropriate thyroid gland function and its hormone activity are crucial in animals. Changes in blood thyroid hormone concentrations are indirect indications of changes in thyroid gland activity (Todini, 2007). The concentrations of these hormones are affected by many factors including season, nutrition, age, gender, climate, breed, ovarian endocrine function, physiological factors (i.e., pregnancy, lactation, and reproduction), and other diseases (Nazifi *et al.,* 2007; Todini, 2007 and Huszenicza *et al.,* 2002). These hormones are the most important factors involved in setting and modulating the basal metabolic rate in target tissues, such as liver, heart, kidney, and brain (Chatterjea and Shinde, 2005; Saicic *et al.,* 2006). Thyroid hormones ($T_3$ and $T_4$) affect lipid metabolism through increasing lipolysis in adipose tissue and stimulating lipogenesis by increasing the activities of some enzymes (Eshratkhah *et al.,* 2008). The serum cholesterol level generally varies inversely with thyroid activity (Bruss, 2008), but there are some contradictory findings regarding the relation between serum thyroid hormones, cholesterol and triglycerides, as well as the concentrations of thyroid hormones were not correlated with cholesterol levels in some other animals (Nazifi *et al.,* 2007 and Nazifi *et al.,* 2000). The net effect of thyroid hormones on cholesterol metabolism is increasing at a rate of cholesterol catabolism by the liver (Bruss, 2008).

Tail chasing may be associated with serum cholesterol elevations in dogs. High serum cholesterol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol levels may be used as biochemical parameters of compulsive tail chasing in clinical settings (Yalcin *et al.,* 2009). It is reported that changes in concentrations of thyroid hormones in fallow-deer, sheep and hamsters are due to the effect of temperature and season (Bruss, 2008). In a research by Tuckova,1995, the levels of cholesterol and total lipids were determined by the BIO-LA-tests (Lachema, Brno, Czech Republic). During the observed period, cholesterol and total lipids concentrations ranged within the reference values in German shepherd and Dachshund breeds (Tuckova *et al.*, 1995). To the best of our knowledge, there is little previous report on the correlation of thyroid hormones with serum lipids and lipoproteins (cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol) in dogs. Therefore, this study was undertaken to investigate the relationship between thyroid hormones with the serum lipids and lipoproteins in clinically healthy German shepherd dogs in Ahvaz district, Iran. The effects of season, sex and age were also reviewed.

## Materials and methods

This study was performed on blood samples from sixty clinically healthy German shepherd dogs in Ahvaz district, southwest of Iran (12 meter above sea level) in 2011. The entire period of investigation was classified into two seasons, from July to September (Average temperature 34.3ºC, maximum temperature 50.8ºC and minimum temperature 22ºC) and from January to March (Average temperature 19.9ºC, maximum temperature 32.4ºC and minimum temperature 4ºC ). The studied dogs

were of both sexes, German shepherd breed with different ages (less than and above three years) and were selected randomly. Sampling was done only once from each animal in every season. In the present survey, we focused on dogs aged from 1.5–5.5 years in order to have a homogenous group of healthy animals whose serum thyroid concentrations could not be influenced by young or old ages. Twenty eight male and thirty two female dogs were vaccinated against DHPPi-L vaccines (Hipra dog- Spain) and were treated with Praziquantel forte (Alfasan Woerden Holland) approximately three weeks before the study. Twenty six of the studied dogs were below three years and thirty four were above three years. This study was performed under the control of the Iranian Society for the Prevention of Cruelty to Animals. The studied dogs were non-pregnant and free from internal and external parasites. The age of the animal was estimated using dental formulary. All animals were fed a chicken and rice diet during the research period and had free access to water. After clinical examination, cephalic blood samples were collected in plane tubes, free from anticoagulant. The serum samples were separated after centrifugation at 750g for 10 min and stored at -20 C until analysis. The samples with haemolysis were thrown away. The levels of cholesterol, triglyceride (T.G), and lipoproteins (HDL-, LDL-, and VLDL-cholesterol) were determined by methods described previously (Nazifi *et al.*, 2007). The levels of serum thyroxine ($T_4$) and triiodothyronine ($T_3$) were measured weekly by radioimmunoassay (RIA) method (commercially available human kits from Immunotech Company, Immunotech-Radiova, Prague, Czech Republic) in the Jahad-Daneshgahi Research Center, Ahvaz, Iran. The validation for these hormones assays considered limits of detection, precision of standard curve following sample dilution and intra- and inter-assay coefficient of variation results.

*Statistical analysis*

Dogs were grouped by age, gender and season. The obtained data were analyzed by SPSS 16.0 statistical software (Illinois, Chicago). Two sample t-tests were used to detect differences in the parameters between the two sexes. Correlations of each of the serum lipids and lipoproteins with the thyroid hormones were analyzed by Pearson's correlation tests. Analysis of Variance (ANOVA) tests were used to compare the serum lipids and lipoproteins between the different age groups of native dogs. All values shown as mean±standard error (SE) and data with $p<0.05$ was seen as statistically significant.

## Results

The mean serum concentrations of cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol (mmol/L), T4 and T3 (µmol/L) were 165±8.78, 113±18.46, 83.46±6.39, 64.68±9.26, 22.42±3.59, 1.48±0.14 and 0.79±0.72 respectively. The level of above parameters in two summer and winter seasons were 1.72±1.12, 84.36±13.16, 69.36±4.89, 89.32±9.82, 16.48±2.32, 1.47±0.14, 0.69±0.69 and 1.58±5.17, 142±19.86, 97.56±6.18, 40.04±4.76, 28.36±3.97, 1.49±0.14, 0.92±0.68 respectively. The measured serum concentrations of cholesterol, triglyceride, HDL-cholesterol, LDL cholesterol and VLDL-cholesterol in the studied dogs have been shown in Tables 1, 2 and 3. Season had a significant effect on T.G, HDL, LDL and VLDL levels in the studied dogs ($p<0.05$). The serum concentrations of $T_3$ and $T_4$ were lower in summer compared with winter season, without significant difference ($p>0.05$). No significant differences were detected for the measured cholesterol, T3 and T4 in different age, sex and season groups. Correlation coefficients between the measured parameters (thyroid hormones, lipids, and lipoproteins) are presented in Table 4.

**Table 1. Concentrations of lipids and lipoproteins in the serum of clinically healthy dogs in different age groups (mean±SE)**

| Age | Number | Cholesterol (mmol/L) | Triglyceride (mmol/L) | HDL (mmol/L) | LDL (mmol/L) | VLDL (mmol/L) | $T_3$ (μmol/L) | $T_4$ (μmol/L) |
|---|---|---|---|---|---|---|---|---|
| < 3 years | 26 | 163.77±6.83 | 111.58±16.12 | 79.65±5.32 | 62.42±8.81 | 22.15±3.21 | 0.87±0.75 | 0.14±0.72 |
| > 3 years | 34 | 166.92±10.71 | 115.33±19.67 | 87.58±7.13 | 67.12±9.62 | 22.71±3.74 | 0.73±0.07 | 1.56±0.13 |

## Discussion

In the present study, the serum concentrations of cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, T3 and T4 are reported for the first time in clinically healthy German shepherd dogs in Ahvaz district, Iran. As expected, our results about serum concentrations lipids and lipoproteins showed that season had a significant effect on T.G, HDL, LDL and VLDL levels (Table 3), but there was not a significant difference with cholesterol. As shown in Table 3, the level of cholesterol was 172.40±1.12 in summer and 158.16±5.17 in winter. It's been previously reported that the serum cholesterol level generally varies inversely with thyroid activity (Bruss, 2008). According to our results, the serum concentrations of both $T_3$ and $T_4$ were decreased in summer compared with winter season, without significant difference. Some authors believe that during summer the exposure of animals to the high environmental temperature depressed the functional activity of the thyroid gland and thereby caused a relatively lower concentration of thyroid hormones. Cold environment may be a stimulus to increase the thyrotrophic hormone output thereby resulting in a higher concentration of thyroid hormones in serum. However; there are some evidences to suggest conversely, that thermal exposure acts directly on the hypothalamic pituitary axis and is caused a reduction in TSH secretion (Chatterjea and Shinde, 2005). Although the role of thyroid hormones are well known in many species, there is little evidence describing probable relationship between thyroid hormones status, and serum profiles of lipid and lipoproteins (cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol) in dogs. It was reported that the serum levels of $T_3$ and $T_4$ changed with season in German shepherd and Dachshund dogs, where they had the lowest levels in summer and the highest level of $T_3$ in winter and $T_4$ in autumn (Tuckova *et al.,* 1995). In another survey by Korhonen 1987 the levels of thyroid hormones ($T_3$, $T_4$), total lipids and urea in blood serum of adult farmed raccoon dogs were monitored year-round, and compared with seasonal changes in body weight and feed consumption during intense, maintenance and restricted fasting feeding. Thyroid hormone levels tended to be low during winter, but no marked seasonal differences were observed during the rest of the year. The colder the temperature, the less the animals consumed the feed supplied. Marked seasonal changes in body weight of the animals were found. This marked seasonal change was mainly the result of changes in subcutaneous fat reserves (Korhonen 1987). Monitoring of basal TSH concentrations in different months did not reveal seasonal dependency (Oohashi *et al.,* 2001). Kaciuba-Ubcilko *et al.*,1975 reported changes in serum total thyroxine concentration and the effects of thyroxine and 3,5,3'-triiodothyronine injection on plasma free fatty acid level and rectal temperature responses in six dogs at rest and during 1 h of submaximal treadmill exercise. During exercise, 5 hour after a single T4 injection (0.1 mg/kg), there was a significant increase in $TT_4$, although the resting level was markedly elevated (Kaciuba-Ubcilko *et al.,* 1975). The highest concentrations of total thyroxine occur in puppyhood and that a marked decline in its

concentration occurs from 3 to 9 years of age, with a subsequent rise in $TT_4$ concentration (Ferm *et al.,* 2009). Many authors have been described specific differences in $TT_4$ and $fT_4$ concentrations in different breeds of dogs. Studies of Greyhounds have shown that they have basal $TT_4$ concentrations below the non-breed-specific reference range, and that $fT_4$ concentrations are also relatively low (Hill *et al.,* 2001). Similar results were also found in Whippets, where the mean $TT_4$ values were significantly lower in comparison with the control group, but no significant differences were seen between Whippets and control dogs for $fT_4$ and TSH (Geffen *et al.,* 2006). In the present study, the serum concentrations of the measured lipids and lipoproteins were somewhat different from the previously reported ranges for dogs. The explanation for these findings is unknown at this moment and may be due to the factors such as age, sex, hydration, health status, breed, heat stress and pregnancy on serum lipids and lipoproteins profile. A serum concentration of insulin was compared between gender-matched hypothyroid and healthy client-owned dogs within comparable age and body condition score ranges by Mazaki-Tovi M *et al*., 2010. Insulin concentration was significantly higher in the hypothyroid compared to normal dogs following adjustment for potential confounders. Their study showed that canine hypothyroidism is associated with increased serum insulin concentration, neither of which may be attributed to obesity alone (Mazaki-Tovi *et al.,* 2010). In another survey by Verkest *et al.,* 2012, serum triglyceride concentrations were measured before and hourly for 12 hours after a meal. Fasting triglyceride concentration was not significantly associated with pancreatic lipase immunoreactivity concentrations. None of the dogs with high triglyceride concentrations and one of the dogs with low fasting and peak postprandial triglyceride concentrations developed clinically important pancreatic disease (Verkest *et al.,* 2012). There is no previous information about effect of season on the serum lipids and lipoproteins of dogs. In the current study, the effective factors such as breed and pregnancy were omitted and non-pregnant dogs were studied.

**Table 2. Concentrations of lipids and lipoproteins in the serum of clinically healthy dogs in different gender groups (mean±SE)**

| Sex | Number | Cholesterol (mmol/L) | Triglyceride (mmol/L) | HDL (mmol/L) | LDL (mmol/L) | VLDL (mmol/L) | $T_3$ (µmol/L) | $T_4$ (µmol/L) |
|---|---|---|---|---|---|---|---|---|
| Female | 32 | 169.03±8.42 | 104.90±13.25 | 81.21±5.50 | 65.28±46.64 | 20.90±2.63 | 0.81±0.07 | 1.58±0.11 |
| Male | 28 | 160.10±9.16 | 125.10±23.66 | 86.57±7.28 | 63.86±9.86 | 24.52±4.54 | 0.80±0.07 | 1.33±0.17 |

**Table 3. Concentrations of lipids and lipoproteins in the serum of clinically healthy dogs in different season groups (mean±SE)**

| Season | Number | Cholesterol (mmol/L) | Triglyceride(mmol/L) | HDL(mmol/L) | LDL (mmol/L) | VLDL (mmol/L) | $T_3$ (µmol/L) | $T_4$ (µmol/L) |
|---|---|---|---|---|---|---|---|---|
| summer | 30 | 172.40±1.12 | 84.36±13.16 | 69.36±4.89 | 89.32±9.82 | 16.48±2.32 | 0.69±0.07 | 1.47±0.14 |
| winter | 30 | 158.16±5.17 | 142.40±19.86 | 97.56±6.18 | 40.04±4.76 | 28.36±3.97 | 0.92±0.07 | 1.49±0.14 |

Another study by Choi *et al.,* 2011 provides basic data on physiological and hematological characteristics in beagle dogs. In the serum biochemical profiles, alkaline phosphatase was slightly higher in males than females, while the total cholesterol of female dogs at 9-months-old was higher than that of males at the same age. Other biochemical components, including alanine aminotransferase, blood urea nitrogen, creatinine, triglyceride, and total protein were non-significantly increased with age in both sexes (Choi *et al.,* 2011). There are some contradictory findings compared with results of other researchers that had worked on

the serum concentrations thyroid hormones with the cholesterol and triglyceride concentrations in other animals such as sheep or goat (Nazifi *et al.*, 2007; Eshratkhah *et al.*, 2008). Daily rhythmicity of lipid metabolism occurs in some animals (Bertolucci *et al.*, 2008). Only the rhythmic pattern of triglycerides responded to a 6h delay in light onset, suggesting a cardinal role of a light-entrained circadian oscillator on its generation. Rhythms of total lipids, total cholesterol, phospholipids, and triglycerides vanished when dogs were food-deprived, indicating that these rhythms are driven by the digestive process (Bertolucci *et al.*, 2008). In another survey, 127 dogs were selected during routine visits. Total cholesterol and triglycerides were found to be higher in obese dogs with respect to normal weight dogs. Thyroid hormones may be involved in the regulation of fatty acid delta-6-desaturase activity in dogs (Pena *et al.*, 2008). The effects of thyroid hormones on the serum and cutaneous fatty acid concentration profiles of dogs were evaluated by Campbell and Davis (1990). Thyroidectomized dogs had significant increases in serum oleic acid and linoleic acid concentrations. These changes were reversed in response to thyroid hormone replacement. Similar changes were found in cutaneous fatty acid concentration profiles (Campbell and Davis, 1990). Chemistry tests were conducted on serum from young Beagle dogs in order to determine the effect of age on these parameters by Wolford *et al.*, (1988). Rapidly changing age related differences were observed in several parameters. Cholesterol, triglycerides, lactate dehydrogenase, thyroxine, glucose, gamma glutamyl transpeptidase, and total bilirubin values were elevated early in life, and decreased during the first 6 to 8 weeks. Thyroxine attained stability by three months (Wolford *et al.*, 1988). To the best of our knowledge, there was no previous research regarding the correlation of the thyroid hormones with the serum lipids and lipoproteins in dogs in Iran.

**Table 4. Correlation coefficients and statistical analysis between the measured parameters in the serum of clinically healthy dogs for total samples (n=60)**

| | $T_4$ | $T_3$ | Cholesterol | TG | HDL | LDL | VLDL |
|---|---|---|---|---|---|---|---|
| $T_4$ | | | | | | | |
| Pearsons correlation | 1 | 0.440** | 0.224 | 0.153 | 0.091 | 0.093 | 0.114 |
| Sig. (2-tailed) | | 0.001 | 0.117 | 0.287 | 0.529 | 0.521 | 0.429 |
| $T_3$ | | | | | | | |
| Pearsons correlation | | 1 | 0.181 | 0.345* | 0.302* | - 0.211 | 0.330* |
| Sig. (2-tailed) | | | 0.209 | 0.014 | 0.033 | 0.141 | 0.019 |
| Cholesterol | | | | | | | |
| Pearsons correlation | | | 1 | -0.119 | 0.287* | 0.737** | -0.152 |
| Sig. (2-tailed) | | | | 0.411 | 0.044 | | 0.292 |
| Triglyceride | | | | | | | |
| Pearsons correlation | | | | 1 | 0.107 | - 0.411** | 0.952** |
| Sig. (2-tailed) | | | | | 0.461 | 0.003 | |
| HDL | | | | | | | |
| Pearsons correlation | | | | | 1 | -0.169 | 0.044 |
| Sig. (2-tailed) | | | | | | 0.241 | 0.763 |
| LDL | | | | | | | |
| Pearsons correlation | | | | | | 1 | -0.430** |
| Sig. (2-tailed) | | | | | | | 0.002 |
| VLDL | | | | | | | |
| Pearsons correlation | | | | | | | 1 |
| Sig. (2-tailed) | | | | | | | |

* Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)

As a general, the cause of these findings and some contradictory findings regarding the relation between serum thyroid hormones and lipid and lipoproteins are not clear in different

animals. Geographic and dietary factors may affect the serum concentrations of lipid and lipoproteins in the animals and more research is required on a larger number of animals, before the importance of these findings can be assessed.

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# غلظت‌های سرمی لیپیدها و لیپوپروتئین‌ها و ارتباط آنها با هورمون‌های تیروئیدی در سگ‌های ژرمن شپرد سالم از نظر بالینی: اثر فصل، جنس و سن

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## چکیده

اگرچه یافته‌های زیادی در زمینه ارتباط بین هورمون‌های تیروئیدی سرم، کلسترول، تری‌گلیسرید و لیپوپروتئین‌ها در حیوانات مختلف وجود دارد، اما منابع در سگ‌ها محدود می‌باشند. هدف از انجام این مطالعه، تعیین ارتباط بین هورمون های تیروئیدی و غلظت سرمی پروفایل‌های لیپیدی و لیپوپروتئین‌ها و نیز تعیین ارتباط بین پارامترهای اندازه‌گیری شده در گروه‌های مختلف از نظر فصل، جنس و سندر سگ‌های منطقه اهواز، جنوب غرب ایران بود. نمونه‌های خون از ورید سفالیک ۶۰ قلاده سگ سالم از نظر بالینی و غیر آبستن در دو فصل (تابستان و زمستان) و در دو گروه سنی (کمتر و بیشتر از ۳ سال) گرفته شدند. نمونه‌برداری از هر حیوان، تنها یک‌بار انجام شده بود (۳۰ قلاده در هر فصل). غلظت‌های سرمی کلسترول، تری‌گلیسرید، VLDL، LDL، HDL و ارتباط آنها با هورمون‌های تیروکسین ($T_4$) و تری‌یدوتیرونین ($T_3$) در سگ‌های مورد مطالعه اندازه‌گیری شدند. در تحقیق حاضر، میانگین غلظت سرمی کلسترول، تری‌گلیسرید، HDL، LDL، VLDL، $T_4$ و $T_3$ اندازه‌گیری شدند. پارامترهای فوق در دو فصل تابستان و زمستان نیزبدست آمدند. فصل اثر معنی‌داری بر مقادیر تری‌گلیسرید، HDL، LDL و VLDL در سگ‌های مورد مطالعه داشت ($p<0.05$). غلظت سرمی $T_3$ و $T_4$، به شکل غیر معنی‌داری در فصل تابستان کمتر از زمستان بود ($p>0.05$). هیچ تفاوت معنی‌داری برای مقادیر کلسترول، $T_3$ و $T_4$ در گروه‌های مختلف از نظر سن، جنس و فصل وجود نداشت ($p>0.05$). به دانش ما، هیچ‌گونه مطالعات قبلی در زمینه ارتباط بین هورمون‌های تیروئیدی با لیپیدها و لیپوپروتئین‌های سرمی در سگ‌های ایران وجود ندارد. نتایج حاضر نشان داد که هورمون‌های تیروئیدی بر مقادیر تری‌گلیسرید، HDL، LDL و VLDL تاثیرگذار بوده و بخشی ازتغییرات فصلی مشاهده شده در سرم، ممکن است ناشی از تغییر در هورمون‌های تیروئیدی باشد.

**واژگان کلیدی:** تیروکسین ($T_4$)، تری‌یدوتیرونین ($T_3$)، لیپید، لیپوپروتئین، سگ، اهواز